Microbial Pretreatment of Corn Stover for Improved Enzymatic Saccharification and Ethanol Production

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Abstract

Microbial pretreatment is considered to be an environmentally friendly and energy saving method to remove lignin from lignocellulosic biomass for improved cellulose conversion. In this study, pretreatment of corn stover with the white rot fungus *Ceriporiopsis subvermispora* was found to preferentially degrade lignin with a limited loss of cellulose. The maximum selectivity (defined as the ratio of lignin degradation over cellulose degradation) reached 6.16 after 35 days of pretreatment. Microscopic observation indicated that the cell wall was modified by fungal attack. The treated corn stover resulted in significant improvement of enzymatic hydrolysis and ethanol yield, which was about four times higher than that of non-treated corn stover. No inhibitor to ethanol fermentation was formed during microbial pretreatment. This is the first report of microbial pretreatment of corn stover for its conversion to ethanol by selective delignification with *C. subvermispora*, which has great potentials for developing an economically feasible and environmentally benign pretreatment process for ethanol production from lignocellulosic biomass.

Introduction

Rising energy demand and global warming have inspired worldwide research and development of biofuels, especially from lignocellulosic materials. Corn stover has the greatest potential to be used for cellulosic ethanol production with a projected availability of 170~256 million dry tons annually in the United States (Perlack et al. 2005). The goal of this research was to develop a cost-effective and environmental friendly pretreatment process to reduce the recalcitrance of corn stover for ethanol production.

The hypothesis was that microbial pretreatment of corn stover by *C. subvermispora* can selectively degrade lignin and significantly improve hydrolysis and ethanol yield. The specific objectives of this project were to (1) study the degradation of cell wall components during the microbial pretreatment and (2) study the effect of fungal treatment on cellulose digestibility and ethanol fermentation yield of corn stover.

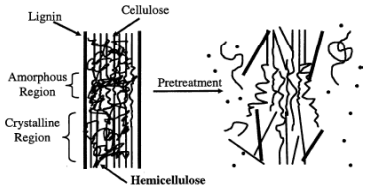


Figure 1. Schematic of goals of pretreatment on lignocellulosic biomass (Mosier et al. , 2005)

Materials and Methods

Corn stover was obtained from a local farm of Wooster, air-dried and ground to 5 mm for pretreatment. Ground corn stover was mixed with DI to obtain a moisture content of 75% and then inoculated with *C. subvermispora* and statically incubated at 28°C for up to 35 days. Samples were taken every 2-7 days for compositional analysis, enzymatic hydrolysis, and ethanol fermentation.

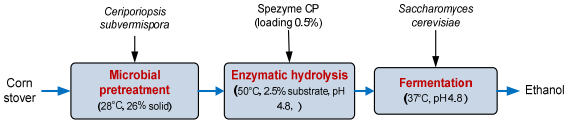


Figure 2. Diagram of ethanol production from corn stover with microbial pretreatment

Results

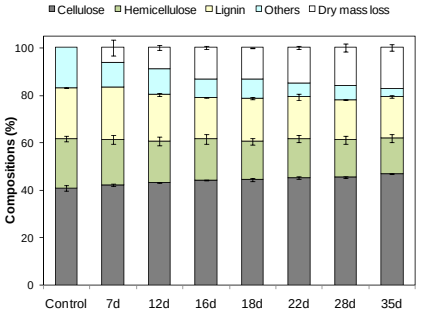


Figure 3. Compositional change of corn stover during microbial treatment.

Increase in the cultivation time caused an increase in cellulose content , but decrease in hemicellulose and lignin as well as total dry mass in the treated corn stover.

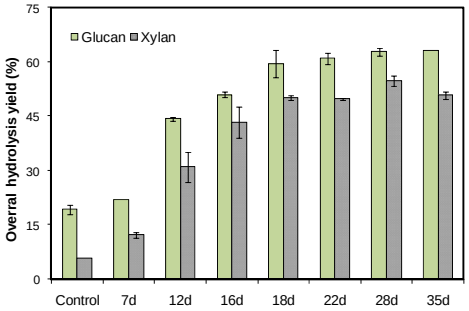


Figure 4. Enzymatic hydrolysis yield of microbial treated corn stover

The maximum glucose yield of 63.0% was obtained after 28 days of pretreatment , which was 4 times higher than that of untreated corn stover. Moreover, fungal treatment resulted in 10 folds increase in xylan conversion.

Table 1. Selectivity of lignin degradation and cellulose degradation

	Dry mass loss (%)	degradation (%)	Cellulose loss(%)	Hemicellulose loss (%)	Selectivity (%)
Control	3.42±1.88	2.40±1.18	2.28±0.86	1.80±0.00	1.06
7d	6.10±3.36	3.22±0.15	2.73±1.11	14.77±8.41	1.18
12d	8.98±1.09	16.55±3.06	3.75±0.40	23.36±7.75	4.42
16d	13.37±0.59	28.91±0.52	6.41±0.38	27.64±7.85	4.51
18d	13.40±0.16	27.10±1.94	5.78±1.25	32.79±5.44	4.69
22d	14.81±0.67	29.53±5.24	5.56±1.22	33.00±6.01	5.25
28d	15.81±1.59	33.25±1.00	6.05±0.26	36.31±6.62	5.49
35d	17.34±1.46	31.68±2.15	5.14±0.06	41.25±6.97	6.16

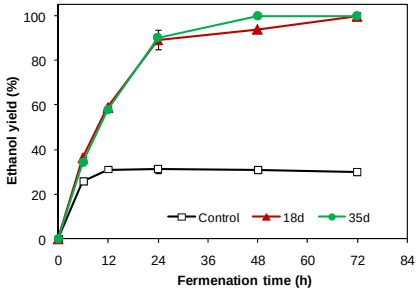
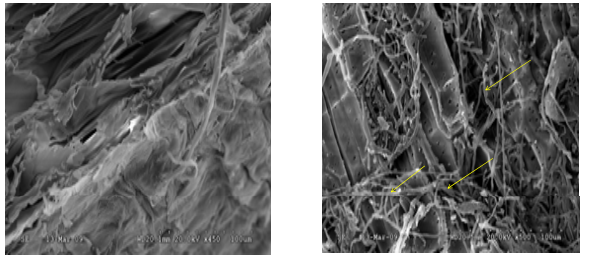


Figure 5 Ethanol yield of microbial treated corn stove

Fungal treatment of corn stover for 18d led to almost complete conversion of glucan to ethanol (99.91%) after 72h fermentation. Increase pretreatment time to 35 days did not cause significant increase in ethanol yield.



(a) Untreated (control), 450x

(b) Microbial treated for 12 days, 500x

(c) Microbial treated for 12 days, 900x

(d) Microbial treated for 12 days, 1000x

Figure 6. Scanning electron micrographs of untreated and microbial treated corn stover

The SEM images of the 12 day-treated samples showed that the fungal hyphae colonized the tracheids of corn stover rapidly and penetrate the cell wall (Figure 4c & d), resulted in cellulose-rich matrix surrounding the hyphae.

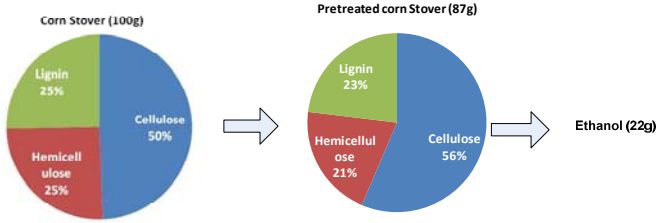


Figure 7. Mass balance of corn stover pretreated by *C. subvermispora*

Conclusions and Discussion

The white rot fungus *C. subvermispora* can colonize the surface of corn stover and penetrate the cell wall (Figure 6c), cause selectively degradation of lignin with limited cellulose loss. The pretreatment can significantly improve enzymatic hydrolysis of cellulose and hemicellulose. Complete conversion of glucan to ethanol on the treated corn stover (18d, 35d) was obtained after 72h fermentation. The microbial ptreatemnt did not produce any fermentation inhibitors.

Compared to thermochemical pretreatment, the microbial pretreatment process developed in this study did not use any chemicals and elevated temperature/ pressure. It can be done at ambient conditions with energy saving. This process can be an effective, economical and environmentally friendly pretreatment for ethanol production from corn stover.

Acknowledgments

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References

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